CLAIMS:

We claim:

1. A method of comparing protein compositions of interest between at least two different samples which comprises:

(a) preparing an extract of proteins from each of said at least two different samples;

(b) providing a set of substantially chemically identical and differentially isotopically labeled protein reagents, one for each sample wherein said reagent has a formula selected from the group consisting of :

A-L-PRG and L-PRG wherein A is an affinity label that selectively binds to a captive reagent, L is a linker group in which one or more atoms are differentially labeled with one or more stable isotopes and PRG is a protein reactive group that selectively reacts with a given protein functional group or is a substrate for an enzyme;

(c) reacting each protein sample of step (a) with a different reagent from said set of step (b) to provide isotopically labeled proteins;

(d) mixing each of said isotopically labeled proteins to form a single mixture of different isotopically labeled proteins;

(e) electrophoresing the mixture of step (d) by an electrophoresing method capable of separating proteins within said mixture; and

(f) detecting the difference in the expression levels of the proteins in the two samples by mass spectrometry based on individual peptides derived from chemical or enzymatic digestion.

2. The method of claim 1 wherein said reagent has the formula:

A-L-PRG

and affinity tagged proteins in the samples are enzymatically or chemically processed to convert them into labeled peptides.

3. The method of claim 1 wherein said reagent has the formula:

L-PRG

and labeled proteins in the samples are enzymatically or chemically processed to convert them into labeled peptides.

4. The method of any one of claims 1, 2 or 3 wherein the protein or peptide portion of one or more of the labeled proteins are sequenced by tandem mass spectrometry to identify the labeled protein from which the peptide originated.

5. The method of any one of claims 1, 2 or 3 wherein the proteins are identified by peptide mass fingerprinting, and the isotopically labeled peptides are used for quantitation.

6. The method of any one of claims 1, 2 or 3 in which the amount of one or more proteins or peptides in the samples is also determined by mass spectrometry and which further comprises the step of introducing into a sample a known amount of one or more internal standards for each of the proteins to be quantified.

7. The method of any one of claims 1, 2 or 3 wherein the released isotopically labeled proteins or peptides are separated by chromatography prior to detecting and detection by mass spectrometry.

8. The method of claims 1, 2 or 3 where the samples consist of protein mixtures derived from tissues, cells, biological fluids including serum, cerebrospinal fluid, urine, ascites, or subcellular fractions including supernatants and various membrane-containing organelles or nuclear preparations, or protein preparations separated by chromatographic methods, capillary electrochromatography or capillary electrophoresis methods.

9. The method of claims 1, 2 or 3 where the proteins are identified by any protein staining technique, or where protein-containing regions are localized by mass spectrometry following systematic digestion and extraction or any combination of transblotting and digestion.

10. The method of any one of claims 1, 2 or 3 in which a plurality of proteins or peptides in one sample are detected and identified.

11. The method of any one of claims 1, 2 or 3 further comprising a step in which one or more of the proteins or peptides in a sample are chemically or enzymatically processed to expose a functional group that can react with a label.

12. The method of any one of claims 1, 2 or 3 wherein PRG is a protein reactive group that selectively reacts with certain protein functional groups and a plurality of proteins or peptides are detected and identified in a single sample.

13. The method of claim 12 wherein two or more substantially chemically identical and differentially isotopically labeled protein reactive reagents having different specificities for reaction with proteins or peptides are provided and reacted with each sample to be analyzed.

14. The method of claim 13 wherein all of the proteins or peptides in a sample are detected and identified.

15. The method of any one of claims 1, 2 or 3 wherein the relative amounts of one or more proteins or peptides in two or more different samples are determined and which further comprises the steps of combining the differentially labeled samples, capturing isotopically labeled components from the combined samples and measuring the relative abundances of the differentially labeled proteins or peptides.

16. The method of claim 1, 2 or 3 which determines the relative amounts of membrane proteins in one or more different samples.

17. The method of claim 15 in which different samples contain proteins originating from different organelles or different subcellular fractions.

18. The method of claim 15 in which different samples represent proteins or peptides expressed in response to different environmental or nutritional conditions, different chemical or physical stimuli or at different times.

19. The method of claim 1 wherein absolute protein concentration is deduced by comparison to a known amount of a deuterated or non-deuterated peptide standard, where this standard was derived by chemical synthesis or was isolated from biological samples.

20. The method of claim 1 whereby multiple samples are labeled with PRG containing different numbers of heavy atoms so that multiple samples can be separated on a single gel and analyzed at one time.

21. The method of claim 1 whereby proteins of special interest that are previously known to be particularly informative are analyzed based on their location on a 1D or 2D gel. These proteins can include disease markers as well as control proteins.

22. The method of claim 1 whereby the post-translational modification status of particular proteins are monitored by gel analysis.